Supplementary Materials

Supplementary Table 1: Patient Characteristics by Ascertainment

Variables	Overall (N=243)	MSK-IMPACT (N=133)	LS Registry (N=110)	p-value[b]
Sex				
Male	96 (40%)	60 (45%)	36 (33%)	0.049
Female	147 (60%)	73 (55%)	74 (67%)	
Age at 1st cancer diagnosis (median, range)[a]				
Male	53.5 (15-84)	55.5 (15-84)	44 (28-74)	0.22
Female	53 (1-88)	53 (1-88)	51 (30-74)	0.59
Self-Reported Race/Ethnicity				
Non-Hispanic White	209 (86%)	107 (80%)	102 (93%)	0.14
Black	14 (6%)	10 (7%)	4 (3%)	
Asian	10 (4%)	8 (6%)	2 (2%)	
Other	2 (1%)	2 (2%)	0 (0%)	
Hispanic	5 (2%)	2 (2%)	1 (1%)	
Unknown	3 (1%)	4 (3%)	1 (1%)	
BMI at study enrollment, kg/m^2 (median, range)				
Male	26.7 (17.6-39.8)	26.8 (17.6-39.8)	26.7 (21.5-36.8)	0.78
Female	25.3 (15-52.8)	25.7 (15-52.8)	23.9 (17.9-42.9)	0.36
Smoking Status				
Ever smoker	67 (28%)	42 (32%)	25 (23%)	0.93
Never smoker	142 (58%)	89 (67%)	53 (48%)	
Unknown/Missing	34 (34%)	2 (1%)	32 (29%)	

[a] In cancer-affected patients only.
[b] p-values are chi-squared/fisher's exact or ranksum.

Legend: Table depicts clinical characteristics for all patients overall and by ascertainment (MSK-IMPACT vs. LS Registry)

Supplementary Table 2: Detailed Histology of Various Cancer Types

A.

Endometrial Cancer Histology N=53		
MSH6	Overall (n=41)	MMR-D/MSI-H (N=25)
Endometrioid	31	20
Clear Cell	3	1
Mixed	2	2
Leiomyosarcoma	1	1
De-differentiated	0	0
Missing	4	1
PMS2	Overall (n=12)	MMR-D/MSI-H (N=10)
Endometrioid	7	7
Clear Cell	1	0
Mixed	2	2
Leiomyosarcoma	0	0
De-differentiated	1	1
Missing	1	0

B.

Ovarian Cancer Histology N=17		
MSH6	Overall (n=5)	MMR-D/MSI-H (N=2)
Endometrioid	1	0
Clear Cell	1	1
Mixed endometrioid and serous	1	0
Carcinosarcoma	1	1
Sertoliform/Wolffian	1	0
PMS2	Overall (n=12)	MMR-D/MSI-H (N=4)
Endometrioid	3	2
Clear Cell	4	0
High grade serous	3	1
Poorly Differentiated	1	1
Sex Cord Stromal	1	0

C.

Skin Tumor/Cancer Histology N=11		
MSH6	Overall (n=6)	MMR-D/MSI-H (N=3)
Sebaceous adenoma	4	3
squamous cell carcinoma	1	0
Missing	1	0
PMS2	Overall (n=5)	MMR-D/MSI-H (N=0)
Basal Cell Carcinoma	3	0
Squamous Cell Carcinoma	2	0

Legend: Tables depict detailed histological data on endometrial (A), ovarian (B), and skin (C) tumors/cancers observed in our cohort, overall and in MMR-D/MSI-H tumors.

Supplementary Table 3: Age at Diagnosis of LS-associated Cancers with Screening/Risk-Reduction Recommendations

	MSH6				*PMS2*			
Cancer Type	All Tumors (N)	Age (median, range)	MMR-D/MSI-H Tumors (N)	Age (median, range)	All Tumors (N)	Age (median, range)	MMR-D/MSI-H Tumors (N)	Age (median, range)
Colorectal	47	50 (27-78)	33	50 (27-73)	31	54 (29-80)	23	54 (29-80)
Endometrial	41	55 (39-66)	25	55 (39-66)	12	56.5 (49-74)	10	57 (49-74)
Ovary	5	55 (40-56)	2	55.5 (55-56)	12	69 (37-73)	4	52.5 (45-73)
Gastric/Esophageal	5	66 (44-72)	3	69 (44-72)	1	33 (33)	0	
Pancreas/Biliary	5	69 (64-88)	3	69 (67-72)	7	70 (36-81)	1	63 (63)
Urothelial	5	61 (42-84)	4	69.5 (42-84)	2	47 (28-66)	1	66 (66)
Small Bowel	1	76 (76)	1	76 (76)	5	47 (36-51)	5	47 (36-51)
Skin Tumors, Non-melanoma	6	52 (35-65)	3	58.5 (52-65)	5	64 (61-67)	0	

Legend: The table depicts number and age at diagnosis (median and range) for Lynch Syndrome-associated cancers with screening recommendations.

Supplementary Table 4: Clinical Details of Patients with Colorectal Cancers Diagnosed Age <35

Study ID	Total Tumors	Sex	Gene	Pathogenic Variant	Age at 1st Cancer	MMR-D/ MSI-H CRC	IHC results[b]	Somatic Profile	Meets criteria for CMMRD[c]? (Y/N)
53	5 - Urothelial - Myxopapillary Ependymoma - CRC x 3	Male	*PMS2*	c.1687C>T (p.Arg563*)	28	Y	PMS2 absent	N/A	N (1 CALM)
88	1	Female	*MSH6*	c.3476dupA (p.Tyr1159*)	31	Y	MSH6 absent	49 variants (Not Ultrahypermutated[a])	N
92	1	Male	*PMS2*	Deletion of exon 10	30	Y	PMS2 absent	N/A	N
94	1	Female	*PMS2*	c.765C>A (p.Tyr255*)	30	Y	PMS2 absent	N/A	N
120	1	Female	*MSH6*	c.3573dupT (p.Val1192Cysfs*2)	32	Y	MSH6 absent	70 variants (Not Ultrahypermutated[a])	N
162	1	Male	*MSH6*	c.1569_1570delTT (p.Tyr524Glnfs*6)	31	Y	MSH2 and MSH6 absent	420 variants (Ultrahypermutated[a])	N
243	1	Female	*MSH6*	Deletion exons 3-9	27	Y	MSH2 and MSH6 absent	90 variants (Not Ultrahypermutated[a])	N

Legend: Table depicts clinical characteristics, tumor somatic profiling results, and criteria for constitutional mismatch repair deficiency (CMMRD).
[a] Ultrahypermutated defined as >150 somatic variants
[b] Immunohistochemistry results, absence only noted in tumor tissue whereas normal tissue demonstrated intact expression
[c] Proposed diagnostic criteria via C4CMMRD.[1,2]
Abbreviations: CRC – colorectal, Y – Yes, N- No, CALM - café-au-lait macules. N/A: Not available
For IHC results, if not specified then the protein was retained.

Supplementary Table 5: Additional P/LP Germline Variants in LS Heterozygotes

MSK-IMPACT (N=133) Other P/LP Variants				
Gene	*Variant*	*ACMG/AMP Classification*[b]	*LS Gene*	*Cancers*
RTEL1	c.3791G>A (p.Arg1264His)	P/LP	MSH6	Lymphoma, Esophageal SCC (MMR-D/MSI-H)
MUTYH	c.1187G>A (p.Gly396Asp)	P/LP	PMS2	Pancreatic cancer (MSS)
BRCA2	c.6468_6469delTC (p.Gln2157Ilefs*18)	P/LP	PMS2	**Pancreatic cancer (MSS)**
APC	c.3920T>A (p.Ile1307Lys)	P/LP	MSH6	Urothelial cancer (MMR-D/MSI-H), Prostate cancer
RECQL4	c.1048_1049delAG (p.Arg350Glyfs*21)	P/LP	PMS2	Endometrial Cancer (unknown MSI/MMR status), CRC (MSS/MMR proficient)
APC[a]	c.3920T>A (p.lle1307Lys)	P/LP	MSH6	CRC (MMR-D/MSI-H)
FH[a]	c.1431_1433dupAAA (p.Lys477dup)	P/LP	MSH6	CRC (MMR-D/MSI-H)
RECQL	c.1138A>T (p. Lys380*)	P/LP	PMS2	Ovarian Cancer (MSS/MMR proficient)
MUTYH	c.536A>G (p.Tyr179Cys)	P/LP	MSH6	CRC (MMR-D/MSI-H)
RAD51D	c.574C>T (p.Gln192*)	P/LP	PMS2	**Ovarian Cancer (MSS/MMR proficient)**
SDHA	c.667delG (p.Asp223Ilefs*3)	P/LP	PMS2	Ovarian Cancer (MSS/MMR proficient)
FH	c.1431_1433dupAAA (p.Lys477dup)	P/LP	MSH6	Gastric cancer (MMR-D/MSI-H)
CHEK2	c.1283C>T (p.Ser428Phe)	P/LP	MSH6	**CRC (MSS/MMR proficient)**
BAP1	c.783+2T>C ()	P/LP	PMS2	CRC (MMR-D/MSI-H)
APC	c.3920T>A (p.Ile1307Lys)	P/LP	MSH6	Endometrial cancer (MMR-D)
LS Registry (N=110) Other P/LP Variants				
Gene	*Variant*	*ACMG/AMP Classification*[b]	*LS Gene*	*Cancers*
BRCA2	c.6468_6469delTC (p.Gln2157Ilefs*18)	P/LP	PMS2	Unaffected
APC	c.3920T>A (p.Ile1307Lys)	P/LP	MSH6	Unaffected

[a] Represents same patient with 2 variants
[b] ACMG/AMP classification of variants[3]

References:

1. Wimmer K, Kratz CP, Vasen HFA, et al. Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'Care for CMMRD' (C4CMMRD). *Journal of Medical Genetics*. 2014;51(6):355-365. doi:10.1136/jmedgenet-2014-102284
2. Aronson M, Colas C, Shuen A, et al. Diagnostic criteria for constitutional mismatch repair deficiency (CMMRD): recommendations from the international consensus working group. *Journal of Medical Genetics*. 2021:jmedgenet-2020-107627. doi:10.1136/jmedgenet-2020-107627
3. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in medicine : official journal of the American College of Medical Genetics*. May 2015;17(5):405-24. doi:10.1038/gim.2015.30